Journal of Medical Virology 59:91–97 (1999)

Comparison of Cytomegalovirus Antigenemia and Culture Assays in Patients On and Off Antiviral Therapy

Kirsten St. George* and Charles R. Rinaldo, Jr.
Clinical Virology Laboratory, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania

We examined 1,869 consecutive blood specimens from 529 patients (>80% organ transplant recipients) for detection of CMV by antigenemia and culture assays, and compared results between patients on and off antiviral therapy. All 1,869 specimens were tested by the shell vial assay and antigenemia, and 503 were also tested by standard tube culture. The overall positivity rate for each test was 17.0% for antigenemia, 1.8% for shell vial culture assay, and 0.7% by tube culture. No specimens were positive by either shell vial or tube culture, while negative by antigenemia. These findings were consistent across all organ transplant and other patient types. Shell vial positivity was associated with higher antigenemia levels in patients either on or off anti-CMV drug therapy. Among the shell vial positive specimens, the antigenemia counts were higher in patients on antiviral drug therapy as compared to those not on therapy. We conclude that the pp65 antigenemia assay is superior to culture methods for detection of CMV in blood, particularly for patients on anti-CMV drug treatment. Additionally, its quantitative nature renders the antigenemia assay an excellent tracking tool for both resolution of asymptomatic, low level CMV reactivations and response of CMV infection to antiviral treatment. ***J. Med. Virol. 59:91–97, 1999.***

KEY WORDS: transplantation; viral load; virus quantitation; infectious disease

INTRODUCTION

Cytomegalovirus (CMV) is still the major viral cause of infectious disease complications in transplant recipients [Dauber et al., 1990; Rubin, 1990; Kramer et al., 1993; Petri, 1994; Sable and Donowitz, 1994; Fishman and Rubin 1998], resulting in increased cost and length of hospital stay [McCarthy et al., 1993; Falagas et al., 1997] and predisposing patients to other serious infections [Falagas et al., 1996; George et al., 1997]. Therefore improved methods for the detection and quantitation of CMV infection are important in the monitoring and management of these patients. The pp65 antigenemia assay has been reported to be a sensitive test for the detection of CMV in peripheral blood [The et al., 1990, 1992; Pérol et al., 1993]. The sensitivity of the antigenemia assay is optimized by the use of freshly collected specimens for testing [Niubò et al., 1994; Landry et al., 1995], formalin rather than acetone fixation [Gerna et al., 1992; Landry and Ferguson, 1993; Boeckh et al., 1994], immunostaining for pp65 rather than p72 or p150 antigens [Grefte et al., 1992; Gerna et al., 1993], and the use of immunofluorescence rather than immunoperoxidase staining techniques [Jiwa et al., 1989; Boeckh et al., 1992; Gerna et al., 1992; Landry and Ferguson, 1993; Myers and Amsterdam, 1997].

Although most studies have found the CMV antigenemia assay to be more sensitive than culture assays [van der Bij et al., 1988; Revello et al., 1989; Boland et al., 1990; van den Berg et al., 1991; Boeckh et al., 1992; Erice et al., 1992; Gómez et al., 1992; Halwachs et al., 1993; Mazzulli et al., 1993; Hebart et al., 1996; Wetherill et al., 1996; Brumback et al., 1997], some reports assess it as comparable in sensitivity to the shell vial assay or tube culture [Lipson et al., 1993,1994; Schmidt et al., 1994; Lipson et al., 1997]. Moreover, numerous studies find it to be clinically useful for diagnosing and monitoring CMV infection in patients [Gerna et al., 1993; Koskinen et al., 1993; Boeckh et al., 1996; Eriksson et al., 1996; Niubò et al., 1996; Lo et al., 1997; Murray et al., 1997; Nicholson et al., 1997]. In contrast, the detection of CMV nucleic acid by the polymerase chain reaction (PCR) on blood has been found to be useful in some studies [Ehrnst et al., 1995; Einsele et al., 1995; Stéphan et al., 1997; Roberts et al., 1998], whereas others have found it to be too sensitive [Delgado et al., 1992; Weber et al., 1994; Lo et al., 1997]

*Correspondence to: Kirsten St. George, Clinical Virology, Room A-912, UPMC Presbyterian, 200 Lothrop Street, Pittsburgh, PA, 15213. E-mail: stgeorgek@msx.upmc.edu

Accepted 16 March 1999

and some studies detect positive samples in large percentages of healthy blood donors [Bevan et al., 1991; Taylor-Wiedeman et al., 1991; Zhang et al., 1995; Krajden et al., 1996]. Our own "in-house" semiquantitative CMV PCR for DNA in leukocytes, while showing a temporal relationship between time to positivity and likelihood of progression to disease [Manez et al., 1996], also proved to be too sensitive for clinical utility [Koehler et al., 1996]. Our efforts have therefore concentrated on the CMV antigenemia assay.

In this study, the sensitivity of the antigenemia assay was compared to that of both the shell vial culture assay and standard tube culture for detection of CMV in blood specimens from a large cohort of predominantly organ transplant recipients. Additionally, the effects of antiviral therapy on test outcome were examined, since we have shown previously that drug treatment can affect the results of CMV culture assays [Mañez et al., 1994], and others have reported a loss of sensitivity in culture assays but not antigenemia, in patients on antiviral drug therapy [Gerna et al., 1990; Boeckh et al., 1992; Erice et al., 1992; Landry et al., 1993; Storch et al., 1994].

MATERIALS AND METHODS

Patients and Specimens

Consecutive blood specimens (1,869) from 529 patients were received for CMV testing. These comprised specimens from liver transplant recipients (1,128 specimens), renal transplant recipients (269 specimens), lung transplant recipients (19 specimens), heart transplant recipients (18 specimens), small bowel transplant recipients (12 specimens), multi-organ transplant recipients (129 specimens), bone marrow transplant recipients (103 specimens), and others (191 specimens). The median patient age was 46 (range 3–76) and median number of specimens per patient was 1 (range 1–24).

At the beginning of this study, standard tube culture, shell vial assay and antigenemia testing were performed on all blood specimens received for CMV testing. After the first 503 specimens had been tested however, standard tube culture was discontinued from the routine procedure for CMV detection in blood. Hence the following 1,366 specimens were only tested by shell vial assay and antigenemia.

For analysis of the effects of antiviral therapy on the outcome of CMV assays, patient charts from organ transplant recipients with positive test results were retrospectively examined. A total of 318 specimens from 113 patients (90 solid organ transplant recipients, 7 bone marrow transplant recipients, 5 HIV positive patients, and 11 others) were CMV positive by at least one assay. For this section of the analysis, efforts were concentrated on the 90 solid organ transplant recipients. Some of these patients had returned to their home country or state soon after transplant, and post-transplant treatment information was therefore incomplete. Complete treatment data for the study period were available on 63 of the solid organ transplant recipients: 41 livers, 11 kidneys, 5 hearts, 2 lungs, and 4 multi-organs. Anti-CMV drug treatment information was correlated with CMV shell vial and antigenemia test results.

CMV Assays

Leukocytes were prepared from each peripheral blood specimen by dextran sedimentation and the concentration adjusted to 1×10^6 cells per ml in Hanks balanced salt solution. Aliquots of 0.2 ml (200,000 leukocytes) were inoculated into each of two shell vials containing coverslips with MRC-5 human fetal lung fibroblast monolayers (Bio-Whittaker, Walkersville, MD) and processed for the CMV shell vial assay as previously described [Gleaves et al., 1984] with minor modifications [St. George and Rinaldo, 1994]. In specimens where standard tube culture was also performed, replicate 0.2 ml aliquots of the leukocyte preparation were inoculated into each of two human foreskin fibroblast tube cultures (Bartels, Issaquah, WA) for standard virus culture. All specimens were also tested for CMV by the pp65 antigenemia assay as previously described [Gerna et al., 1992] with minor modifications [St.George and Rinaldo, 1997]. Briefly, aliquots of 0.2 ml (200,000 leukocytes) were loaded into each of three cytospin chambers and centrifuged on a Shandon Cytospin 2 (Shandon, Pittsburgh, PA) at 900 rpm for 3 minutes. Slides were air dried, fixed in formalin, permeabilized with NP40 detergent as previously described, and allowed to air dry. Cytospin preparations were then stored at 4°C overnight and stained the following morning for CMV pp65 by indirect immunofluorescence. Anti-CMV pp65 mouse monoclonal antibody (Clone 1C3, Biosoft, Argene, France) was applied to two cytospin preparations per specimen, while phosphate-buffered saline (PBS) was applied to the third as a control. The slides were incubated at 37°C for 30 minutes in a humidified chamber. After rinsing in PBS, the fixed cells were stained with fluorescein isothiocyanate-labeled goat anti-mouse Fab 2 immunoglobulin (Cappel, Durham, N.C.) at 37°C for 30 minutes. Optimal concentrations for both antibodies had been previously determined by cross titration. Slides were again rinsed with PBS, counterstained for a few minutes with Evans blue dye, rinsed with PBS, and mounted with buffered glycerol. The stained cells were observed with fluorescence microscopy under 400× magnification for the typical green nuclear fluorescence. For each specimen, the saline control was read first to determine if there was any non-specific binding of reagents. Positive cells were counted on each of the two test cytospin preparations for each specimen, and the higher of the two counts was taken as the test result.

RESULTS

As shown in Table I, 683 specimens were tested by antigenemia and shell vial alone, with a positivity rate of 18.2%. Of the 1,186 specimens tested by the three assays, 194 (16.4%) gave a positive result. The overall positivity rate for all specimens was 17.0%. These posi-

TABLE I. CMV Culture and Antigenemia Results on Peripheral Blood Leukocytes

	Tested by AG[a] and SV[b]		Tested by AG[a], SV[b] and TC[c]		Total tests	
Patient type	Specimens	Positives (%)	Specimens	Positives (%)	Specimens	Positives (%)
Liver transplant	616	99 (16.1)	512	98 (19.1)	1,128	197 (17.5)
Renal transplant	13	5 (38.5)	256	45 (17.6)	269	50 (18.6)
Bone marrow transplant	6	1 (16.7)	97	9 (9.3)	103	10 (9.7)
Lung transplant	2	0 (0)	17	2 (11.8)	19	2 (10.5)
Heart transplant	4	1 (25.0)	14	4 (28.6)	18	5 (27.8)
Small bowel transplant	0	0	12	0 (0)	12	0 (0)
Multi-organ transplant	37	16 (43.2)	92	10 (10.9)	129	26 (20.2)
Other	5	2 (40.0)	186	26 (14.0)	191	28 (14.7)
Total	683	124 (18.2)	1,186	194 (16.4)	1,869	318 (17.0)

[a]Antigenemia.
[b]Shell vial assay.
[c]Tube culture.

tive specimens were from 113 patients: 90 solid organ transplant recipients, 7 bone marrow transplant recipients, 5 HIV positive patients, and 11 other patients. Positivity rates varied across the different patient groups and were highest in heart transplant recipients (27.8%). These variations, however, were likely related to the low numbers of specimens in some groups, differences in time post-transplant, and antiviral and immunosuppression treatment regimens.

Of the 683 specimens assessed by antigenemia and shell vial only, 10 were positive in shell vial culture, 114 were positive by antigenemia, and none were positive by shell vial culture while negative by antigenemia (Table II). This gave a percentage positivity for shell vial culture of 1.46% (95% confidence interval 0.7–2.68%), and for antigenemia of 16.69% (95% CI 13.97–19.70%). Of the 1,186 specimens assessed by antigenemia, shell vial, and tube culture, only 23 were positive by shell vial culture, and 13 positive by tube culture, while 194 were positive by antigenemia. Again, no specimens were positive by either of the culture assays, while negative by the antigenemia assay. This gave percentage positivities of 1.94% (95% CI 1.23–2.90%) for shell vial culture, 1.1% (95% CI 0.58–1.87%) for tube culture, and 16.63% (95% CI 14.30–18.59%) for antigenemia.

Positive cell counts in the antigenemia assay appeared variable across the different patient groups (Table III). However, 95% confidence intervals demonstrate few significant differences, and these were again likely related to factors such as the low number of positive specimens in some groups, differences in time post-transplant, and different antiviral and immunosuppressive treatments at the time of testing.

When assay results were analyzed with regard to the antiviral treatment data, patients on therapy had higher antigenemia counts than those not on drug therapy (Table IV). The shell vial assay was positive in patients with higher antigenemia counts, regardless of antiviral therapy. There was no significant difference between the shell vial positivity rate for the antigenemia-positive specimens from patients on anti-CMV therapy, compared to those not on drug (chi-square = 0.123, 1 df, $P = 0.726$). Antigenemia positive cell counts were highest on specimens which were shell vial positive and from patients on drug therapy. Although statistical significance for this trend was not demonstrated, sample numbers were very low, particularly for specimens positive by shell vial from patients on therapy ($n = 5$).

DISCUSSION

These results demonstrate that the majority of CMV positive specimens were detected by antigenemia only, with proportional positivity rates for the antigenemia assay approximately ten-fold higher than those for culture assays. Furthermore, this trend was consistent across all patient groups. Thus our study is in agreement with others that have found the antigenemia assay to be more sensitive than either the shell vial assay or standard tube culture for detection of CMV in blood [Boland et al., 1990; Erice et al., 1992; Halwachs et al., 1993; Mazzulli et al., 1993; Revello et al., 1989; van den Berg et al., 1991; van der Bij et al., 1988]. It is considered preferable to prevent CMV disease, rather than treat it once clinically established, in order to decrease the morbidity and mortality caused by CMV infection in transplant recipients. However, due to the toxicity and cost of currently available anti-CMV drugs, many clinicians are reluctant to use prophylactic therapy routinely in all patients. Previous studies have found culture assays to be insufficiently sensitive for the initiation of preemptive therapy [Einsele et al., 1995]. Our results support the use of the CMV antigenemia assay to guide preemptive therapy of CMV infections which we [Grossi et al., 1996; Koehler et al., 1996] and others [Vlieger et al., 1992; Gondo et al., 1994; Locatelli et al., 1994; Gerna et al., 1995] have found to be successful in reducing the incidence of CMV disease.

The positivity rates by shell vial assay and tube culture in this study were surprisingly low. Indeed these rates are lower than in previous studies from our laboratory [St. George and Rinaldo, 1994; Mañez et al., 1994]. This could be related to alterations in the conditions of the current assays, such as the relatively low inoculum of 200,000 leukocytes per tube or vial. Larger cell inoculums provide enhanced sensitivity in both shell vial and tube assays [Buller et al., 1992].

We believe however, that the main reason for the low shell vial and tube culture positivity rate was the effect

TABLE II. Analysis of Results of CMV Positive Specimens

	Tested by AG[a] and SV[b]			Tested by AG[a], SV[b] and TC[c]							
Patient type	AG^+ SV^+	AG^+ SV^-	AG^- SV^+	AG^+ SV^+ TC^+	AG^+ SV^+ TC^-	AG^+ SV^- TC^+	AG^+ SV^- TC^-	AG^- SV^+ TC^+	AG^- SV^+ TC^-	AG^- SV^- TC^+	Total positives
Liver transplant	8	91	0	3	9	5	81	0	0	0	197
Renal transplant	0	5	0	1	1	0	43	0	0	0	50
Bone marrow transplant	0	1	0	1	0	0	8	0	0	0	10
Lung transplant	0	0	0	0	0	0	2	0	0	0	2
Heart transplant	0	1	0	0	2	0	2	0	0	0	5
Small bowel transplant	0	0	0	0	0	0	0	0	0	0	0
Multi-organ transplant	2	14	0	2	1	1	6	0	0	0	26
Other	0	2	0	0	3	0	23	0	0	0	28
Total	10	114	0	7	16	6	165	0	0	0	318

[a]Antigenemia.
[b]Shell vial assay.
[c]Tube culture.

TABLE III. CMV pp65 Antigenemia Quantitation on Positive Specimens

	Number of positive cells/200,000 leukocytes		Number of positive specimens
Patient type	Median	Range	
Liver transplant	6	1–872	197
Renal transplant	15	1–263	50
Bone marrow transplant	16	1–100	10
Lung transplant	689	59–1,319	2
Heart transplant	80	2–811	5
Small bowel transplant	0	N/A	0
Multi-organ transplant	19	1–477	26
Other	26	1–3,328	28
Total	10	1–3,328	318

TABLE IV. CMV Antigenemia and Shell Vial Results in Patients On and Off Anti-CMV Therapy

	Antigenemia positive cell counts[a]		
	No anti-CMV therapy	Ganciclovir therapy	*P* value
Shell vial negative	4 (1–633) (n = 94)	28 (1–1,256) (n = 38)	$P = 0.018$[b]
Shell vial positive	15 (1–811) (n = 15)	120 (1–700) (n = 5)	$P = 0.500$[b]
P values	$P = 0.002$[b]	$P = 0.686$[b]	

[a]Median (range).
[b]Wilcoxon Signed Rank Test for related samples.

of preemptive antiviral treatment. Until recent years, prophylactic antiviral treatment was commonly used at this medical center for prevention of CMV disease in transplant recipients . However, with the changes in treatment practices, most of the patients in this study received preemptive ganciclovir therapy based on CMV antigenemia positivity, rather than drug prophylaxis. Antigenemia positivity usually precedes either shell vial or tube culture positivity, triggering the initiation of antiviral treatment. During the era of prophylactic therapy, we showed a disassociation of the shell vial and tube culture positivity rates during antiviral therapy, with the shell vial positivity rate remaining higher during periods of therapy [Mañez et al., 1994]. Additionally it is generally recognized that antiviral treatment renders isolation of virus more difficult. In this study, conducted mostly after the initiation of preemptive therapy, we suspected a similar effect, with antigenemia positivity remaining higher during antiviral therapy than culture, reducing the relative positivity of shell vial and tube culture, as compared to antigenemia.

To confirm this, we retrospectively analyzed patient charts for antiviral drug treatment data and correlated these with test outcome for the CMV shell vial and antigenemia assays. There was no significant difference among antigenemia-positive specimens between shell vial positivity rates from patients on or off anti-CMV drug treatment. However, when further analyzed by antigenemia positive cell count, shell vial cultures were only positive in patients on drug therapy if the pp65 antigen positive cell count was higher than that observed in patients off drug, whether shell vial negative or positive. The lack of statistical significance is

believed to be due to the low sample number. This suggests that for patients on anti-CMV therapy, culture assays will remain negative until viral load reaches a relatively high level. Furthermore, once placed on antiviral therapy, culture assays will tend to be negative, requiring the antigenemia assay for sensitive monitoring of response to treatment.

In conclusion, we find the CMV antigenemia assay superior in sensitivity to either the shell vial assay or standard tube culture, and that this trend is exacerbated in patients on antiviral therapy. This has important implications with regard to the use of preemptive rather than prophylactic antiviral treatment for CMV, especially in transplant recipients at high risk for CMV disease. Additionally, with the effects of antiviral drug treatment on subsequent CMV culture sensitivity, we find the pp65 antigenemia assay to be the current test of choice for detection of CMV in peripheral blood.

ACKNOWLEDGMENTS

The authors thank Dr. Richard Day and Dr. Qasim Al-Shboul for advice and assistance with statistical analyses, Dr. Elena Percivalle and Dr. Guiseppe Gerna for their assistance with development and implementation of the antigenemia assay, Ardith Mauck for data management, the staff of the Clinical Virology Laboratory, University of Pittsburgh Medical Center for technical assistance, and Sandra Schubert for secretarial assistance.

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